TUMOR VIRUSES

I. Recommended Readings:

D.W. Allen and P. Cole, Viruses and Human Cancer, New England Journal of Medicine 286:70-82, 1972.

J. Tooze, The Molecular Biology of Tumour Viruses, esp. chapter 1, also chapters 6, and 8-10, Cold Spring Harbor Laboratory, 1973.

Review of Medical Microbiology, chapter 40.

Davis et al., Microbiology, chapter 63.

Symposium on Cancer Research, Proceeding of the National Academy of Science 69:1009-1064, April 1972.

II. Topic Outline for Lecture

The evidence for a role for viruses in the etiology of human tumors remains sparse: epidemiological studies do not suggest that conventional, horizontally-transmitted infectious agents are important in human cancer, and virus particles or virus-specific proteins or nucleic acids have only rarely been found in human tumors. Some examples of such findings and their significance will be considered in the herpes virus lecture and in the seminar on tumor viruses.

This lecture is intended to acquaint you with the very rapid advances now being made in the understanding of how viruses cause cancers in animals other than man. Since tumor viruses have been found repeatedly in many species, it seems reasonably likely that they will also be found in man as well; but a profound understanding of the molecular behavior of tumor viruses may be required to know how to look for human agents. Whether or not human cancer is virus-related, animal tumor viruses offer an important opportunity to learn how cells work and how their behavior can be altered by a very small number of viral genes.

General considerations

- RNA versus DNA tumor viruses
- The lysogeny model and circular DNA as a final common pathway
- Biological effects of tumor viruses
- Permissive and nonpermissive cells
- Mechanisms of virus rescue: cell fusion
  helper virus
  chemical induction

DNA tumor viruses

- Classification: herpes viruses, adenoviruses, papova viruses
- Simian virus 40: Life cycle in permissive (monkey) and nonpermissive (rodent) host cells
  organization of the viral genome
  possible role of viral T antigen in transformation
  SV-40 and other papovaviruses in the human population

RNA tumor viruses

General characteristics and species distribution:
- diploid RNA genome
- RNA-directed DNA polymerase ("reverse transcriptase")

Major models:
- provirus mechanism
- oncogenes and virogenes

Endogenous viruses

Rous (avian) sarcoma virus:
- life cycle in permissive host
- organization of the viral genome
- defective viruses
- helper function
- analysis of the transforming gene
- origin of the virus from cellular genes

The search for RNA tumor viruses in human cancer

III. SLIDES (OR EXPANDED VERSONS OF THEM) FROM THE LECTURE

RELEVANCE OF LYSOGENY MODEL TO TUMOR VIROLOGY

VIRUS	GENOME	CIRCULAR dsDNA PHASE	INTEGRATION	LIMITED EXPRESSION OF VIRAL GENES
LAMBDA PHAGE	LINEAR dsDNA	+	+	+
SV40	CIRCULAR dsDNA	+	+	+
AVIAN SARCOMA VIRUS	LINEAR ssRNA	+	+	(+)

DNA TUMOR VIRUSES: GENERAL PROPERTIES

Three size classes:

small (DNA MW ~ 3×10^6)---papovaviruses (SV40, polyoma, papilloma viruses)

medium (DNA MW ~25×10^6)---adenoviruses (from several animal species, eg, chickens, monkeys, man)

large (DNA MW ~100×10^6)---herpesviruses (from several animal species, eg, frogs, chickens, monkeys, man)

Generally enter lytic, replicative cycle with cells from natural host

Transform foreign, nonpermissive host cells with low efficiency. Transformed cells do not produce virus, but virus can be rescued by cell fusion with permissive host.

Only herpes viruses commonly oncogenic in natural host

ANIMALS IN WHICH RNA TUMOR VIRUSES HAVE BEEN OBSERVED

Viper

Chicken (Rous sarcoma, avian leukosis viruses)
Turkey (reticulo-endotheliosis virus)
Pheasant

Mouse (leukemia, sarcoma, and mammary tumor viruses)
Hamster
Rat
Cow
Sheep
Guinea pig
Pig
Cat (leukemia, sarcoma, and RD/CCC viruses)

Baboon
Wooley monkey
Gibbon
Rhesus monkey

PREDICTIONS OF PRINCIPAL MODELS IN RNA TUMOR VIROLOGY

Provirus Model: Infection followed by synthesis, integration and transcription of virus-specific DNA; these functions required for viral replication and cell transformation.

Oncogene-Virogene Model: DNA coding for viral replication and cell transformation inherited by all cells; expressionof this genetic information can occur under appropriate influences (eg, radiation, chemicals infection, other genes, etc.).

EVIDENCE FOR VIRAL GENES IN NORMAL CELLS

1. Viral antigen (many species)
2. Helper factor which forms pseudotype with replication-defective sarcoma virus (mainly chicken)
3. Virus induction (chemical or physical) and spontaneous virus production in cultured cells (many species)
4. Genetic transmission of oncogenic viruses (murine leukemia and mammary tumor viruses)
5. Detection of virus-specific DNA and RNA in uninfected cells (many species)

ENDOGENOUS RNA VIRUSES

1. Genetically transmitted
2. May be induced chemically
3. Often xenotropic
4. Occasionally oncogenic

PRINCIPAL CHEMICAL AND STRUCTURAL FEATURES OF RNA TUMOR VIRUSES

Characteristic shape and budding pattern

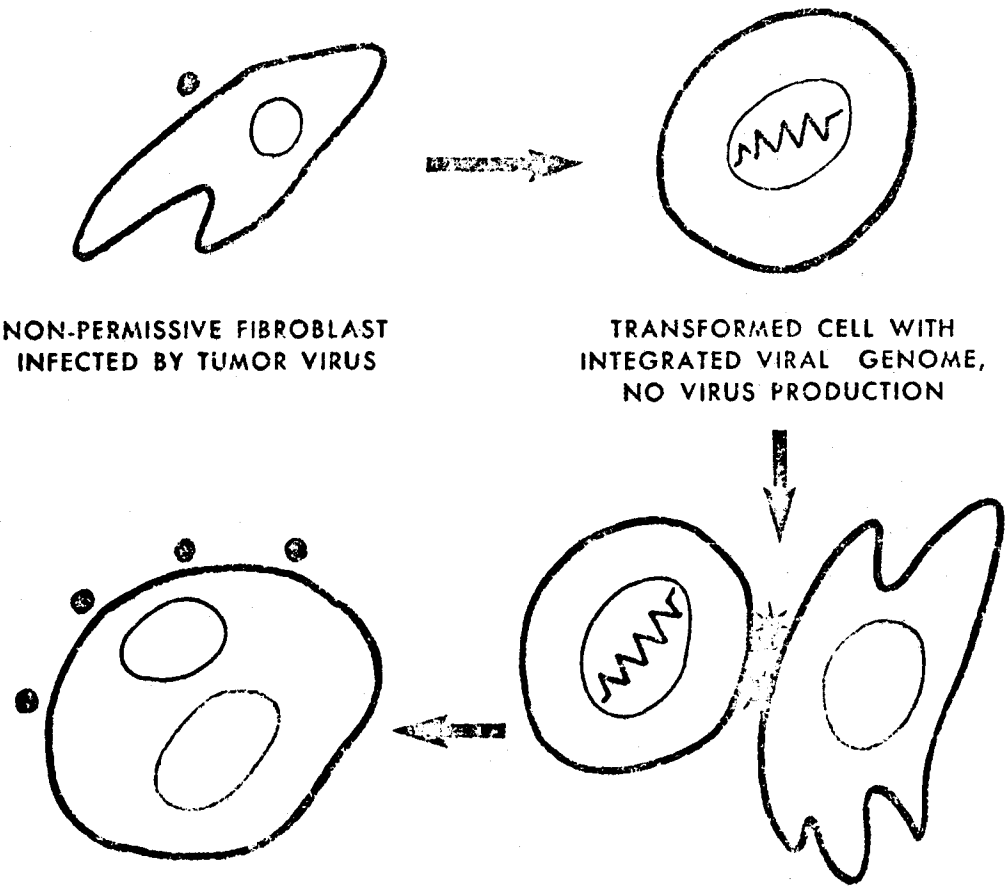
Buoyant density (1.16 for virus, 1.23 for core)

Large, segmented RNA genome (MW 10^7, 2-4 subunits of 3×10^6)

RNA-directed DNA polymerase

Group-specific and type-specific antigens

RESCUE OF TUMOR VIRUS FROM NON-PERMISSIVE HOST

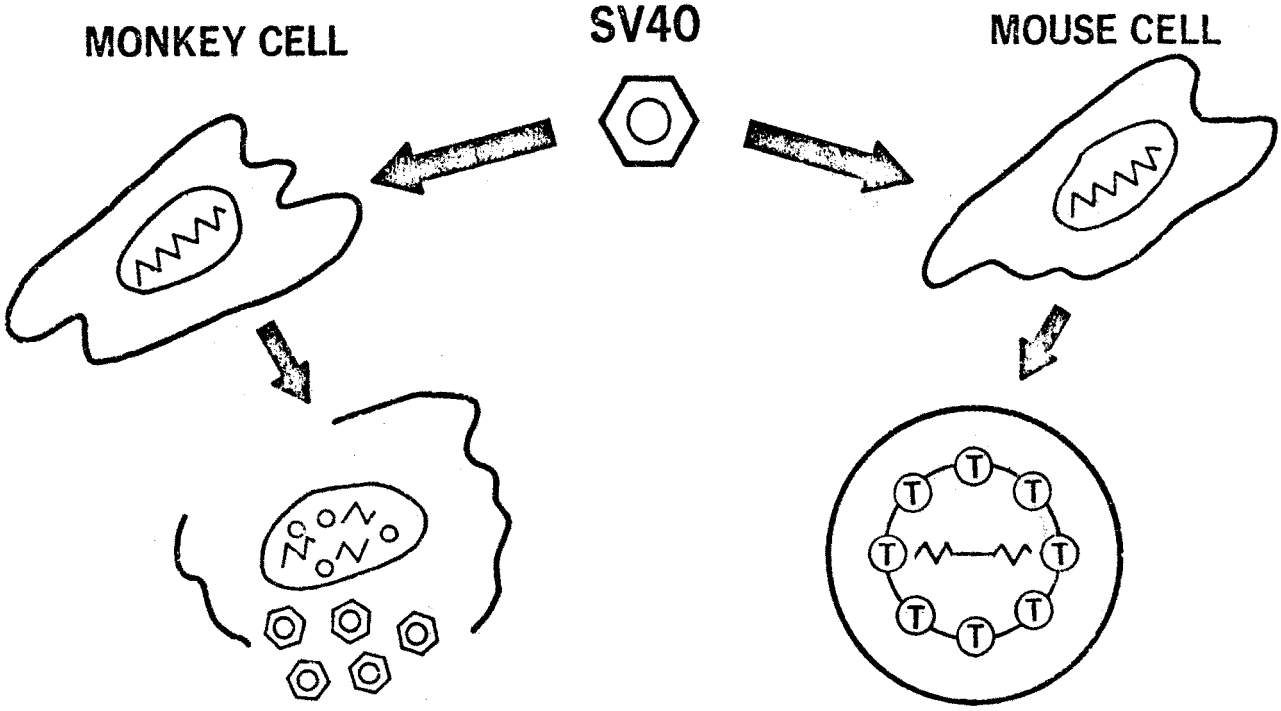


NON-PERMISSIVE FIBROBLAST INFECTED BY TUMOR VIRUS

TRANSFORMED CELL WITH INTEGRATED VIRAL GENOME, NO VIRUS PRODUCTION

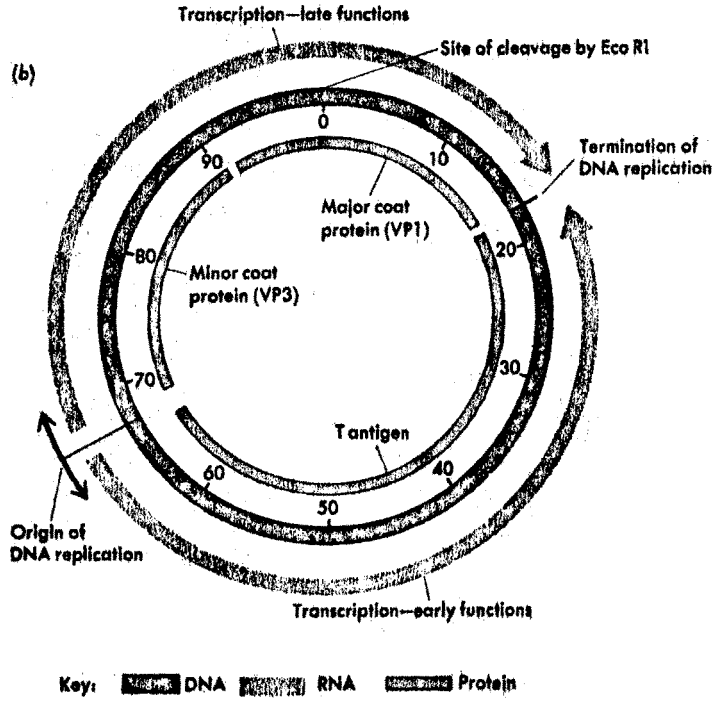
HETEROKARYON PRODUCING TUMOR VIRUS

FUSION OF TRANSFORMED NON-PERMISSIVE CELL WITH NORMAL, PERMISSIVE CELL (paramyxo virus used as fusing agent)

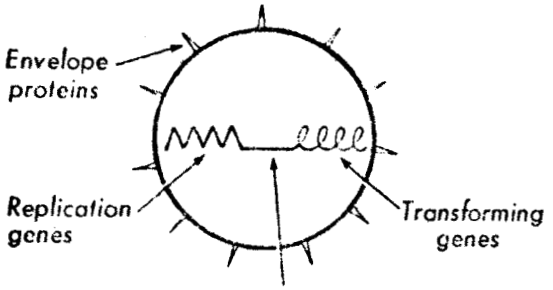


Viral DNA replicated
Early and late genes expressed
Virus produced
Cell lysed

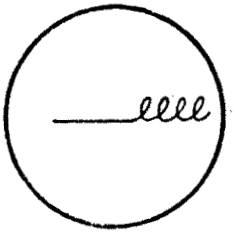
Viral DNA integrated
"Early" gene(s) expressed (T antigen)
No virus produced
Cell Transformed



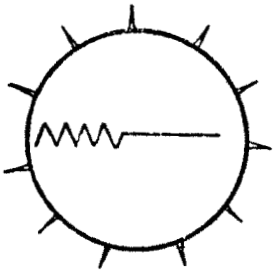
SOME POSSIBLE FORMS OF RNA TUMOR VIRUSES



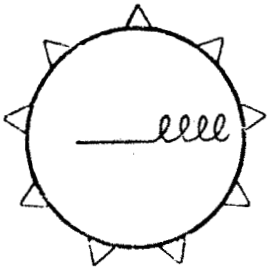
INFECTIOUS
TRANSFORMING
NON-DEFECTIVE



NON-INFECTIOUS
TRANSFORMING
REPLICATION-DEFECTIVE

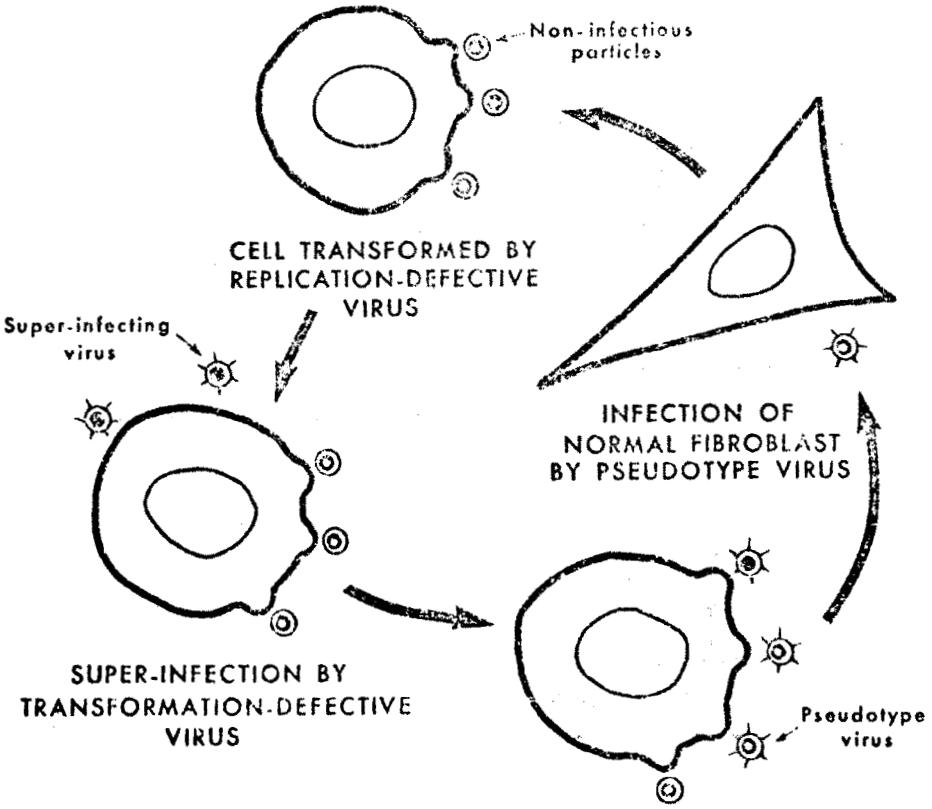


INFECTIOUS
TRANSFORMATION-DEFECTIVE
REPLICATES NORMALLY

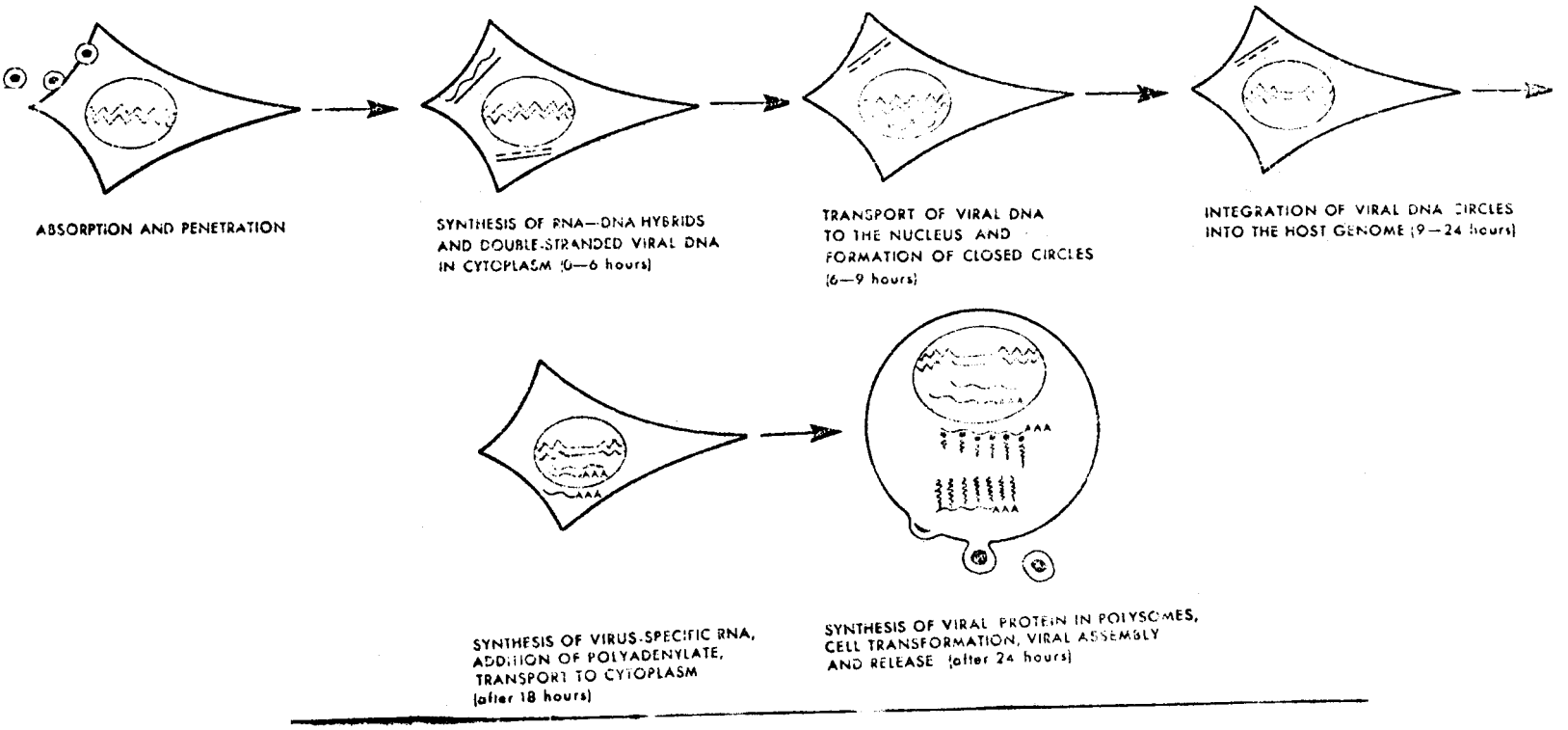


INFECTIOUS (pseudotype)
TRANSFORMING
REPLICATION-DEFECTIVE
(makes non-infectious particles)

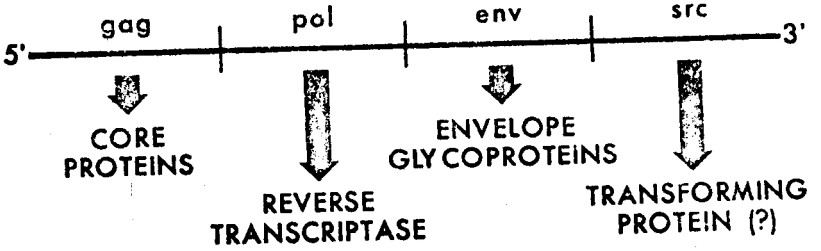
HELPER FUNCTION



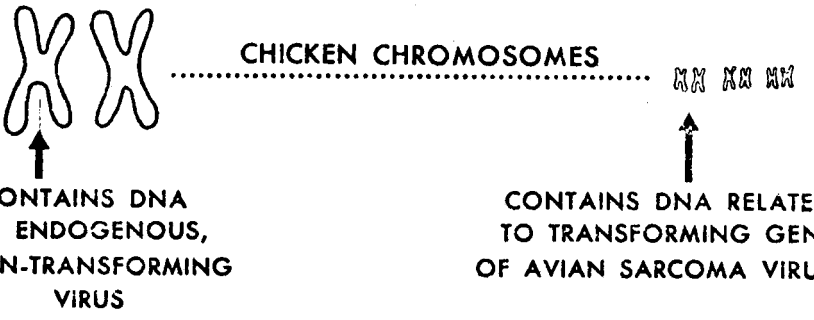
INFECTION OF A PERMISSIVE HOST BY AVIAN SARCOMA VIRUS



GENETIC MAP OF AVIAN SARCOMA VIRUS



ORIGIN OF AVIAN SARCOMA VIRUS



How did these genes recombine?

What is their function in normal cells or in animal cancer?

IV. A Glossary of Tumor Virology

A. General Terminology

Tumor virus---Strictly speaking, a virus capable of causing tumors in vivo; in general, can also transform cells in tissue culture; used loosely to denote viruses which strongly resemble true tumor viruses (e.g., certain induced RNA viruses); also called oncogenic viruses.

DNA tumor viruses---tumor viruses with a genome composed of DNA.

RNA tumor viruses---tumor viruses with a genome composed of RNA.

Permissive host cell---cell which permits replication of a virus.

Nonpermissive host cell---cell which may be infected but does not permit viral replication.

Natural host---animal in which the virus is found in nature (also called "homologous" host); this host is usually permissive.

Heterologous host---animal in which the virus is not found in nature; also called "foreign" host; may be closely or distantly related to the natural host; this host is generally nonpermissive, especially when distantly related.

Vertical transmission---transmission of virus or viral genes from parent to offspring; observed with several RNA tumor viruses, by several mechanisms: genetic transmission (inheritance of tumor virus genes stably associated with the DNA of one or both parents, e.g., endogenous avian and murine RNA viruses); congenital infection (infection of embryo due to infection of a parent, e.g., avian leukemia virus); milk-borne transmission (infectious virus present in breast milk, e.g., murine mammary tumor virus).

Horizontal transmission---occurs between permissive hosts with most DNA and some RNA tumor viruses; but tumors rarely result since the DNA viruses generally are not oncogenic in permissive hosts, and since immunological defences may protect mature animals from consequences of RNA tumor virus infection.

Transformation---alteration of cell behavior in tissue culture; caused by a variety of agents, particularly tumor viruses; can be defined in several ways (change in cell morphology, growth pattern, metabolic phenomena, membrane properties); a transformed cell generally has high tumor-forming capacity when injected into an animal and is considered a laboratory model for a cancerous cell.

Abortive infection---probably a rather common event occurring when either DNA or RNA tumor viruses infect a nonpermissive heterologous host and introduce viral information which is either not expressed or only transiently expressed; in most cases, the viral information is retained by the infected cell; the phenomenon has been described best with papova viruses which cause transient alteration in cell behavior (called abortive transformation).

Reversion---return of transformed cells to normal behavior; observed with cells transformed by either RNA or DNA tumor viruses; in most well-studied examples, the reverted cells retain viral genetic information, but its expression appears to be altered.

Focus---a cluster of transformed cells arising from a viral infection of a single cell; foci produced by a sample of fluid are counted in a common assay for tumor viruses ("focus assay").

Lysogeny model---model based on the mechanism by which certain bacteriophage (eg, lambda phage) insert their genetic information into the DNA of bacteria; predicts that animal tumor viruses can similarly integrate their genes stably into the genome of an infected host cell; the model applies to both RNA and DNA tumor viruses, both of which integrate their genetic information into the host cell genome via a circular DNA molecule.

Integration---the covalent insertion of the genes of tumor viruses into the genome of the host cell.

Heterokaryon---a cell containing nuclei from two different cell types; most commonly formed by fusion of cells of two different species.

Virus rescue by cell fusion---nonpermissive cells transformed by tumor viruses do not synthesize virus; virus can be recovered by fusion of these cells with permissive cells; the heterokaryon releases infectious virus; this phenomenon occurs with both DNA and RNA viruses.

Viral antigens---antigenic proteins coded by a viral genome; they may be virus structural proteins or nonstructural proteins; the latter appear in the infected cell, but not in the virus particle, and include the nuclear "T antigens" of DNA tumor viruses which may be "transforming proteins"; structural antigens of RNA tumor viruses from certain species or groups of species (eg, chickens or mammals) share determinants and are called group-specific (gs) antigens.

B. Terminology Related to DNA Tumor Viruses

Papova viruses---viruses with small, circular (double-stranded) DNA genomes (mol. wt. ca. 3-5 x 10^6); "papova" derived from common members of this group: papilloma viruses (from several natural hosts); polyoma virus of mice; and vacuolating viruses (causes vacuole formation upon infection of natural host) of which simian virus 40 (SV 40) is most famous example.

Papilloma viruses---found in several species (including man) in which they replicate and cause warts (papillomata); grow poorly in cell culture and therefore not well studied.

Polyoma virus---common in wild mice in which the virus occasionally produces a variety of types of tumor (hence "poly" "oma," or many tumors); in cell culture, undergoes lytic, replicative cycle in its natural host (mouse) and transforms certain heterologous, nonpermissive hosts (eg, hamster).

SV 40---discovered in rhesus monkey cells during development of polio vaccine; causes tumors in and transforms cells from certain heterologous, nonpermissive hosts (eg, mouse and other rodents); undergoes lytic, replicative cycle in monkey cells.

Adenoviruses---viruses with medium-sized, linear (double-stranded) DNA genomes (mol. wt. ca. 20-25 x 10^6); found in many species, including man; the several human types show low, medium, or high oncogenicity when used to infect newborn rodents (especially hamsters) or rodent cells in culture (these are nonpermissive, heterologous hosts); replicate in cells from natural host (man) and cause mild, acute GI and respiratory illness.

Herpes viruses---enveloped viruses with large, linear (double-stranded) DNA genome (mol. wt. ca. 100 x 10^6); associated with a variety of diseases, including tumors, in the several hosts in which these viruses have been found (e.g., chicken, frog, subhuman primates, and man); evidence for oncogenicity in man is not conclusive; virus replicates in cells from natural host, and, after irradiation to damage replication genes, it can transform certain heterologous host cells in culture.

Epstein-Barr virus (EBV)---a herpes-like virus originally found in cells from African patient with Burkitt's lymphoma; now commonly seen in human lymphocytes from normal as well as diseased persons; it is the causative agent of infectious mononucleosis and has been indirectly implicated in causation of African Burkitt's lymphoma and nasopharyngeal carcinoma.

C. Terminology Related to RNA Tumor Viruses

Sarcoma viruses---viruses which cause tumors of connective tissue (sarcomas) in natural hosts and (less readily) in foreign hosts; they cause transformation of fibroblasts in cell culture; these viruses replicate in tumor or transformed cells from the natural (permissive) host but not in cells from the heterologous (nonpermissive) host; these viruses have been identified in chickens (Rous sarcoma virus), mice, and cats; they are often defective (unable to synthesize complete infectious virus) and may be accompanied by a helper virus (e.g., leukemia virus) which supplies missing function(s) (e.g., synthesis of envelope proteins).

Leukosis (leukemia) viruses--- strictly speaking, RNA viruses which cause leukemias; these viruses do not ordinarily cause transformation of cultured cells, and the term "leukosis virus" is often applied to any virus which resembles RNA tumor viruses structurally but does not transform cells in culture; these viruses can act as helper viruses for defective sarcoma viruses and are therefore often found mixed with sarcoma viruses; viruses of this type have been observed in many species, particularly chickens, mice, rats, cats, and subhuman primates; some of these are endogenous viruses.

Mammary tumor viruses---RNA viruses which cause mammary carcinoma; the only well-studied example is the murine mammary tumor virus; this virus is either genetically transmitted or milk-borne; it is a B-type particle.

Defective viruses---virus which lack one or more genes required for a given function; replication-defective viruses (e.g., murine sarcoma viruses and some avian sarcoma viruses) lack genes which code for proteins necessary to produce infectious progeny; transformation-defective viruses can replicate normally but lack genes required for transformation.

Helper viruses---viruses capable of supplying the function(s) which replication-defective viruses lack; if a helper virus and a replication-defective virus infect the same cell, some of the progeny virus will include infectious particles with the genome of the replication-defective virus and structural proteins supplied (in part) by the helper virus (such progeny is called pseudotype virus); helper viruses include leukosis viruses.

Virus rescue by helper virus---the recovery of infectious, pseudotype virus from cells infected by a replication-defective virus after superinfection with a helper virus; usually the helper virus and defective virus are native to the same species, but rescue may also occur with viruses belonging to different mammalian species (e.g., feline leukemia virus can rescue the replication-defective murine sarcoma virus).

Endogenous viruses---genetically transmitted RNA tumor viruses; in general, these viruses do not transform cells in culture, are sometimes xenotropic, can act as helper viruses, and can be chemically induced; they have been identified in birds, mice, rats, cats, baboons, and other species; at least two are oncogenic: mouse mammary tumor virus and murine leukemia virus; they afford support for the oncogene-virogene hypothesis.

Xenotropic viruses---viruses which replicate well in foreign hosts and poorly in their natural hosts; these are frequently endogenous viruses.

Induced viruses---RNA tumor viruses which appear in cultures of uninfected cells after treatment with chemical "inducers," particularly halogenated pyrimidines (e.g., BUdR or IUdR); they are prime examples of endogenous viruses.

C-type particle---the morphological designation for the vast majority of RNA tumor viruses; denotes a particle which contains a dense central core (nucleoid) and an envelope with spikes; the core forms at the inner membrane of an infected cell and the envelope is acquired by budding of the virus from the cell membrane.

B-type particle---the morphological designation for a small group of RNA tumor viruses, the principal member being the mouse mammary tumor virus; the core is eccentrically (rather than centrally) located, and the core forms in the cytoplasm (rather than at the cell membrane).

70S RNA---refers to the sedimentation properties of the very high (10×10^6) molecular weight RNA genome characteristic of RNA tumor viruses.

Reverse transcriptase---an enzyme which can transcribe RNA into DNA (i.e., an RNA-directed DNA polymerase); an enzyme of this type is found associated with virtually all RNA tumor viruses, is encoded in the viral genome, and synthesizes the DNA copy of the genome (also called provirus) which integrates into the host cell DNA; enzymes of this type may have some role in differentiation and evolution.

Provirus hypothesis---the proposal that RNA tumor viruses replicate via a DNA intermediate (called a provirus by analogy with prophage); the proposal is now widely accepted due to discovery of the viral enzyme, reverse transcriptase, capable of synthesizing a DNA copy of the RNA genome, and due to direct measurement of viral DNA (provirus) in infected cells.

Oncogene-virogene hypothesis---the proposal that normal, uninfected cells contain, in their DNA, genes coding for RNA tumor virus production ("virogene") or for oncogenicity ("oncogene"); such genes are presumed to be normally unexpressed but activated in response to chemical or physical agents, other viruses, ageing, etc.; the hypothesis is best supported by the presence of endogenous viruses in many species (including chickens, mice, rats, cats, baboons).

V. GENERAL INFORMATION ABOUT AND PROPOSED STRATEGIES FOR IDENTIFICATION OF HUMAN TUMOR VIRUSES---FOR USE IN PREPARATION FOR THE SEMINAR ON TUMOR VIRUSES

If virus particles are not observed in human cancer cells, how can we hope to detect a viral agent which might cause the disease?

1. Seek methods to induce or rescue a virus which for some reason may not replicate in human cancer cells.
2. Seek evidence for occult or incompletely expressed virus. Such "viral footprints" might include viral DNA, RNA, or proteins in tumor cells or antiviral antibodies in sera from tumor-bearing patients.

EVIDENCE IMPLICATING DNA TUMOR VIRUSES IN HUMAN CANCER

1. Argument by analogy: DNA tumor viruses fairly widespread in nature and cause tumors in several species
2. Direct observation in man: papova viruses, adenoviruses, and herpes viruses all found in man, some with great frequency, generally associated with non-neoplastic disease or observed in normal subjects
3. Biochemical evidence:
   a. antibodies to herpes virus antigens in sera of patients with cervical carcinoma
   b. Epstein-Barr virus DNA in cells from Burkitt's lymphoma and nasopharyngeal carcinoma

EVIDENCE IMPLICATING RNA TUMOR VIRUSES IN HUMAN CANCER

1. Argument by analogy: widespread occurrance of RNA tumor viruses in animals
2. Direct observation in man: virus-like particles in human milk, in tumor slices, in cultured cells, or in human placentas (Particles are uncommon, may not be viruses, may not be of human origin, and may be unrelated to cancer.)
3. Biochemical evidence:
   (a) Reverse transcriptase activity associated with high molecular weight RNA and with particles of virus-like density in milk, mammary tumors, leukemic cells, CNS tumors
   (b) In a few cases of leukemia, partial relationship between DNA made by this enzyme and DNA in leukemic cells
   (c) Relationship between RNA in some tumor cells (breast tumors, leukemias, lymphomas) and the RNA genomes of murine tumor viruses
   (d) Relationship between these murine RNA genomes and DNA made by human tumor reverse transcriptase
   (e) Primate and murine virus antigens in some human tumors (also seen in some normal tissues)

MODELS FOR RNA VIRUSES AS HUMAN TUMOR AGENTS

1. Endogenous human tumor virus:
   (a) Might replicate poorly in human cells and better in others
   (b) If xenotropic, might be recovered by fusion of human cells with heterologous cells
   (c) Might be chemically inducible
   (d) Genome would be completely homologous to normal human DNA
2. Human tumor virus analogous to standard animal strains:
   (a) Should either replicate in human cells or be defective in replication functions
   (b) If defective, might be recovered by phenotypic mixing with helper virus
   (c) Likely to be highly infectious for man
   (d) Genome would show complete homology with DNA from tumor, at least partial homology with normal human DNA
3. Tumor virus from another species:
   (a) Unlikely to replicate in human (heterologous) cells unless xenotropic
   (b) If human cells nonpermissive, virus might be rescued by fusion with permissive cells
   (c) Virus probably inefficiently oncogenic in man
   (d) Genome would have homology only with DNA from human tumor tissue (and, of course, with DNA from natural host)
   (e) Tumor cells would contain DNA, RNA, and antigens of animal tumor viruses related to etiological agent

1. EFFECTS OF ANIMALS MODELS ON SEARCH FOR HUMAN RNA TUMOR VIRUSES

Assumption: Virus will be present in tumor cells and will resemble other RNA tumor viruses structurally and chemically.

Pursuit: Search for:
(a) typical particles by electron microscopy,
(b) large RNA in particles of appropriate density
(c) reverse transcriptase activity, especially in association with particles or high molecular weight RNA

2. Assumption: Virus will not replicate in human tumor cells.

Pursuit: Attempt to recover virus by:
(a) chemical induction
(b) fusion with potentially permissive cell
(c) infection with "helper" virus to provide function lacking in tumor virus

3. Assumption: Virus will share nucleic acid sequences or antigenic determinants with known animal viruses.

Pursuit: Seek evidence of viral "footprints" by:
(a) Hybridization of animal virus-specific nucleic acids to DNA and RNA from normal and cancerous human tissues, and
(b) Testing for antigens and antibodies in human tissues and sera with animal virus immunological reagents

HV:mb